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The Determination of Morphine in Opium by Extractions: A New Method

In continuation of the international programme of scientific research undertaken pursuant to resolution 246 F (IX), adopted by the Economic and Social Council on 6 July 1949, the Secretary-General is now publishing a new method for the determination of morphine in opium which has been worked out by the Secretariat.

The Secretary-General wishes to call attention to the fact that the morphine analysis is useful not merely in helping to determine the origin of contraband opium seized from the illicit traffic; it is of paramount importance in the commercial and medical evaluation of licit opium, and in national and international accounting of opium used and drugs produced. It is believed that the Secretariat's new method provides a more accurate standard for the determination of morphine in opium. The Secretary-General suggests, therefore, that it be studied in comparison with the present pharmacopoeial methods to establish the accuracy or degree of error of the latter; and also studied as an assay procedure in its own right.

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THE DETERMINATION OF MORPHINE IN OPIUM BY EXTRACTION: A NEW METHOD

I. SYNOPSIS OF METHOD

1. Stage I: The opium is ground in a mortar with lime and water.

Stage II: The alkaline strength of the filtered lime-water extract is reduced with ammonium chloride and the solution extracted continuously with ether-acetone (a new solvent combination for this purpose) until all the morphine is out. The resulting extract is evaporated to dryness in a dish.

Stage III: The residue is transferred to a separatory funnel with chloroform and dichloroacetic acid solution, and partitioned by shaking out. Dichloroacetic acid solutions in three separatories retain the morphine while chloroform takes out all the other alkaloids. (An entirely new method of separating the other opium alkaloids from morphine.)

Stage IV: The dichloroacetic acid solutions are then combined, made ammoniacal, and the morphine shaken out with chloroform-isobutanol and chloroform-ethanol. The organic solvent extracts are washed and filtered into a dish and then evaporated to dryness.

Stage V: The residue of crystalline morphine is dissolved in a little methyl alcohol, an excess of tenth-normal acid added, using methyl red indicator; the solution diluted with water and back titrated with tenth-normal alkali.

In sum: with the exception of Stage I, (the extraction of the opium with lime-water, for which an aliquot is taken) the extractions are made complete by test. From Stage II onward, it is thought that any negative or positive error in determining the morphine is kept within the equivalent of about 0.1 cc of tenth-normal acid; and thus, it is believed that the objective is essentially realized of separating all the morphine and finally titrating all of it and nothing else.

II. DISCUSSION

2. It is nearly a century and a half since morphine, the first-discovered alkaloid, was originally isolated from opium, but there is still no general agreement as to any uniform "best method" for determining the percentage of morphine in different kinds of opium, nor is any commonly accepted method entirely free from the possibility of substantial, inherent errors. Dozens of chemists have written hundreds of articles on this subject without completely
/solving the

solving the problem. It is, however, believed that the present method is at least a substantial contribution to the subject and indeed in large part a solution of the problem.

3. In an assay, the separation of morphine from lime-water or other aqueous solution, and from the other alkaloids, may be accomplished either by precipitation or by extraction with organic solvents. For the assay of raw opium, precipitation methods are commonly used commercially, and are official in the pharmacopoeias. The "International Method", devised under the auspices of the League of Nations and later accepted by some pharmacopoeias, is such a method. Extraction methods are also used officially in certain cases, when precipitation methods are almost inapplicable; for example, in the assay of camphorated tincture of opium.

4. In spite of the present predominance of precipitation methods for the analysis of opium itself, extraction methods have major points of superiority, assuming that the special difficulties that have beset them can be overcome. Precipitation methods are not flexible: even without considering opium preparations, but only the assay of unmixed raw opium, the results are far from being equally dependable on all types of opium. Furthermore, it is never possible to precipitate all the morphine from a solution; hence a correction must be applied, usually an arbitrary one, or a balancing of errors must be relied on. The precipitated morphine, in the usual methods, is by no means free from other alkaloids. In contrast, an extraction method has a much greater possibility of being applicable to different types of opium with widely varying percentages of morphine, and even to many preparations and mixtures; the extractions can be made complete; and only by an extraction method can we hope to attain the ideal of titrating all the morphine and nothing but morphine.

5. The principal objections that have been brought against previous extraction methods are the following:

(a) That, due to the very limited solubility of morphine in most organic solvents, the sample of opium taken is rather small, increasing the difficulty of getting a representative sample and usually increasing the proportionate effect of impurities.

(b) That in spite of the small sample, large volumes of solvent are used, and the shaking-out of the morphine is excessively difficult and tedious.

/(c) That the

(c) That the residue is frequently too highly coloured.

(d) That, although the non-phenolic alkaloids are separated, the minor phenolic alkaloids remain with the morphine.

6. In the present procedure the opium equivalent (4 grammes) is the same as for the precipitation method of the United States Pharmacopoeia. By means of continuous extraction for Stage II, the objection (a) is met completely and objection (b) in large part. The use of ether-acetone meets objection (c), for this new solvent combination extracts far less colour and other impurities than do solvent-combinations previously used, such as benzene-isopropanol or chloroform-ethanol.

7. The objection (d) that the minor phenolic alkaloids are included with the morphine in extraction processes, although often conceded, has not been nearly as true as has been assumed. Chloroform, as a matter of fact, extracts most of the minor phenolic alkaloids, as well as the non-phenolic alkaloids, from alkali solution. Although this has not been generally recognized, most extractive procedures have utilized back-extraction of the morphine from solution of the alkaloids in chloroform-isopropanol, or some corresponding procedure which separated the greater part of the other phenolic alkaloids from the morphine. When some solvent such as benzene was used to remove the non-phenolic alkaloids from alkali solution the error may have been considerable; with the use of chloroform it can scarcely have exceeded about 0.3 percent of the opium. (This is not to say that previous extraction methods necessarily gave too high results, for there may have been compensating errors in the other direction.) In any case the new method uses instead an extraction from acid which seems to be completely successful.

8. The back-extraction of morphine from an organic solvent solution by means of dilute acid has been used in some previous extractive methods, though probably not for opium assays. Usually it accomplishes little in the way of separating alkaloids; it may or may not result in some separation of the other titratable alkaloids from the morphine, depending on the solvent and the acid in question. In the present procedure, dichloroacetic acid has been chosen as the only acid yet found which, with chloroform, will accomplish the separation desired, and which even makes unnecessary the separation using aqueous alkali.

9. Theoretically, the steps of the new method subsequent to Stage I could be carried out just as well if the aqueous extraction of the opium were made with

/acidified

acidified water instead of lime-water (the solution afterward being made sufficiently alkaline to free the morphine for extraction). In practice there are some difficulties which require further study. Objections have been raised against the use of lime-water for the opium assay, but the most successful and generally accepted methods use it. It has seemed best to work out the rest of the procedure first, and thus provide a sound basis for comparison of this method with the pharmacopoeial and similar assays. In making comparisons, the procedure in Stage I can be modified to correspond exactly, if desired, to the pharmacopoeial assay with which comparison is made. The extraction method has the advantage, however, that there is not the same necessity for limiting the volume of the lime solution, as for a precipitation method.

10. The Secretariat chemists gladly acknowledge a general indebtedness to previous workers in this field. No list of references is given in the present paper; since it is assumed that the interested chemists are acquainted with the basic methods for opium assay, and it is hoped that an extensive bibliography, with commentary, can be offered later.

III. THE METHOD

Stage I. Extraction of the opium with lime-water

11. In a large mortar with pouring lip, grind up 6 grammes of the opium with 20 cc water and 3 grammes calcium hydroxide, $\text{Ca}(\text{OH})_2$. Add 70 cc additional water and stir frequently during half an hour, making sure that finally no opium masses remain undisintegrated. If a sticky layer forms on the pestle, scrape it off with a knife if necessary, and regrind. Pour the lime-water extract through a folded filter and collect 61 cc, representing 4 grammes opium (allowance of 1 cc for extractives).

Stage II. Extraction of the alkaloids from the opium extract

12. The solvent used consists of 150 cc of ethyl ether, $(\text{C}_2\text{H}_5)_2\text{O}$, and 100 cc of acetone, $(\text{CH}_3)_2\text{CO}$. Introduce the lime-water extract to be analyzed, with 250 cc of ether-acetone solvent (150 + 100), into a continuous extraction apparatus (such as that illustrated in Annex A to document E/CN.7/195) with an accompanying boiling-and-receiving flask of 250 to 500 cc capacity; at the same time adding 1.75 grammes ammonium chloride, NH_4Cl , to the lime-water extract. Perhaps the best way is to put a little of the NH_4Cl in the apparatus first, in case a little of the lime-water extract runs through the porous plate. Fill
/the apparatus

the apparatus below the plate with the ether-acetone before pouring in the lime-water extract, and have some of the ether-acetone solvent running into the reception tube of the apparatus, through a folded filter paper, while the extract is poured into the main tube. This procedure will usually keep all of the aqueous solution above the porous plate. Add the rest of the NH_4Cl and rinse in the last drops of the extract, washing down any NH_4Cl clinging to the walls of the main tube, with a few cc water. Keep some ether-acetone solvent running in at the same time. Pour the rest of the ether-acetone solvent into the boiling-and-receiving flask, add a few grains of silicon carbide, SiC , to promote even boiling; connect the apparatus, and turn on the electric hot-plate and the water.

If the porous plate becomes clogged during the extraction, so that the ether-acetone does not rise through it readily enough, the condition is usually corrected easily by adding 2 or 3 cc water down the solvent delivery tube. This can be done through the condenser while the boiling continues.

13. Extract until the morphine is all out. This usually requires at least 4, more often 5 hours, and sometimes considerably longer. To determine if the morphine is all out, shut off the heat, stop or greatly reduce the boiling, and withdraw a test portion of the solvent - about 1 cc - from above the aqueous extract. Evaporate it in a small white porcelain crucible and test with Concentrated Marquis reagent, 9 cc concentrated H_2SO_4 + 1 cc formaldehyde solution (37 - 40%). If the test is not distinctly positive for morphine, another test or tests may be tried, using 3 to 4 cc of the solvent solution at a time, and, if desired, Froehde's and Mecke's reagents as well as Marquis'. (Froehde's reagent: 0.5 gramme sodium or ammonium molybdate in 100 cc concentrated H_2SO_4 . Dissolve with the aid of heat. Contrary to statements in much of the literature, it will keep indefinitely in a glass-stoppered bottle. Mecke's reagent: 0.5 gramme selenious acid in 100 cc concentrated H_2SO_4 .) If the test for morphine still coming out is very weak, and especially if the continuation of the extraction has to go over to the next day, it is best to pour the extract already obtained into an evaporating dish (see the following step, #14), and use a fresh portion of 50 to 150 cc of ether-acetone (60 + 40 ratio) to finish the extraction. In any case, whether the boiling is restarted with the same solvent or fresh solvent, any time the boiling has been completely stopped, add a few grains of SiC before starting it again.

/14. When the test

14. When the test shows no more morphine coming out, pour the liquid in the receiving flask into an evaporating dish weighed to the nearest centigramme. Rinse the receiving flask into the dish with a little methanol; if any morphine has crystallized out in the flask, use hot methanol and rinse two or three times. Evaporate to dryness and then heat for half an hour on the steam bath, in order to drive off volatile bases if any are present. Cool and weigh the dish with its contents before proceeding to the next step. (This weighing is not essential but may yield useful information.)

Stage III. Separation of the other alkaloids from morphine

15. Treat the residue of alkaloids with 15 cc chloroform, CHCl_3 ; cover the dish with a watch-glass, and heat on the steam bath just until the chloroform is hot. Take from steam bath, and, keeping under the hood to avoid breathing the CHCl_3 vapors, loosen the residue with a glass rod. Pour the solution, with as much of the residue as possible, into a separatory funnel.

16. Repeat with 10, 7 1/2, 7 1/2, 5, and 5 cc of CHCl_3 , thus transferring the residue to the separatory funnel with CHCl_3 . (It may not always be necessary to use so many separate portions. A total of 50 cc CHCl_3 is used.) Now add 0.5 cc of pure dichloroacetic acid to the dish. This will dissolve any brownish residue still sticking to the inside of the dish. Add 5 cc water and pour into the same separatory funnel. Rinse out the dish twice more with 5 cc water plus a few drops of dichloroacetic acid each time. Pour into the same separatory funnel.

In two more separatory funnels put 10 and 10 cc water, with two or three drops dichloroacetic acid in each.

17. If any other alkaloids are to be determined from the same extract, two more separatories are necessary (fourth and fifth), containing respectively 8 cc of 5% NaOH solution and 5 cc of 2% NaOH solution. (See part VI for determination of "alkaloid 3" and of any morphine carried out of the acid solutions; and parts VII and VIII for determination of codeine or "porphyroxine-mecnidine".) If only the routine determination of morphine is desired, it is only necessary to interpose a few cc of fresh 2% NaOH solution before each test for completion of the extraction (see paragraph 20).

18. Shake the first separatory thoroughly, and add a small piece of litmus to make sure that the aqueous layer remains acid. Withdraw the CHCl_3 and shake it with the other two dichloroacetic acid solutions in turn; and, if any other

/alkaloids are

alkaloids are to be determined, with the two alkali solutions. The CHCl_3 is then saved for redistillation or for determination of codeine or "porphyroxine-mecnidine".

19. Continue the shake-out with more portions of CHCl_3 , 25 cc each. After five or six shake-outs test a small portion of the CHCl_3 for continued extraction of codeine. When the two alkali solutions are not used, the CHCl_3 , withdrawn from the third acid solution, or part of it, is shaken with a few cc of fresh alkali solution and a small portion taken for the test. Evaporate the test portion in a small white porcelain crucible, cool, and test the residue with concentrated Marquis reagent. A purple colour indicates continued extraction of codeine. If desired, other tests may be made with Froehde's reagent, to be sure of the absence of morphine in the CHCl_3 tested, and with Mecke's reagent, which yields a green colour with codeine. When the two alkali solutions are used, test the CHCl_3 as it is withdrawn from the first alkali solution (fourth separatory). When the Marquis' indication of codeine has become very slight, shake a few cc of the next extract, upon withdrawing it from the last acid solution (third separatory) with a few cc of fresh alkali, and test this CHCl_3 portion with Marquis' and Mecke's reagents for continued extraction of codeine. (This procedure is used because there is a little hold-up of codeine, and more of some other alkaloid yielding a green colour with Mecke's reagent, in the main alkali solution.) The extra portion of alkali may be added to the main alkali solution.

20. Continue until the test for codeine being extracted has become negative or completely negligible. This usually requires some 8 to 14 portions of CHCl_3 . (For further use of the alkali solutions see part VI. For further use of the CHCl_3 see parts VII and VIII.)

Stage IV. Final extraction of the morphine

21. Combine the acid solutions by pouring from the second separatory into the first, and from the third into the second and then into the first, so that all the dichloroacetic solutions are in the first separatory. Then put 5 cc water in the third separatory, shake, and pour it into the second separatory (but not into the first). Put 5 cc more water in the third separatory, shake, and make it ammoniacal with a drop or two of concentrated NH_4OH , then pour it into the second separatory. Finally, add 5 cc water to the third separatory. See that the solutions in the second and third separatories are ammoniacal.

/22. Put in the

22. Put in the first separatory 50 cc of chloroform-isobutanol-methanol solution (30 + 15 + 5). Also have ready 100 cc of chloroform-isobutanol solution (75 + 25). Put a piece of litmus paper in the first separatory and add concentrated NH_4OH , a few drops at a time, until the solution is alkaline to litmus; then add about 1 cc in excess. At once shake vigorously.
23. As soon as there is a good separation and the layers have become nearly clear, withdraw the lower layer from the first separatory into the second, and shake the second separatory. Again, as soon as the layers are well separated, withdraw the lower layer into the third separatory and shake. Then, when the layers have separated, withdraw the lower layer from the third separatory, filtering it through a pledget of cotton in the neck of an ordinary funnel, into an accurately weighed dish which will hold 100 cc of solution.
24. At the same time continue the extraction with the 100 cc of chloroform-isobutanol solution, using 25 cc at a time. When the first two or three extracts (50 and 25, 25 cc) have been run into the dish, put it on the steam bath and evaporate until it will hold more extracts.
25. Run the remaining chloroform-isobutanol extracts into the dish and continue the shakeouts with chloroform-ethanol (85 + 15), 25 cc at a time. Evaporate a little of the first chloroform-ethanol extract from the main solution (first separatory) in a small white porcelain crucible and test the residue with Mecke's reagent. If no green colour is obtained, the aqueous solution in the first separatory can be discarded. (In cleaning this separatory, any sticky brown resinous material remaining in it is easily dissolved by alkali solution.) When the first solution is free of morphine by the Mecke test, a test is made on the extract from the second separatory (with the same or a fresh chloroform-ethanol solution), and finally on the extract from the third separatory. If morphine is still coming out, the extractions are continued with chloroform-ethanol; or if a considerable content of morphine still present is indicated, one or two extractions can be again made with chloroform-isobutanol (3 + 1, ratio), then again with chloroform-ethanol (85 + 15, ratio). (On the other hand, if the morphine content of the opium is known to be quite low, one or two of the chloroform-isobutanol extractions can be omitted, or the volume of the first solvent, the chloroform-isobutanol-methanol, can be somewhat reduced.) The chloroform-ethanol extracts are run into the same dish as the preceding extracts.

/26. Evaporate off

26. Evaporate off the solvent and dry the residue on the steam bath to complete crystallization. Heat for about half an hour more; cool in the desiccator and weigh. Multiplication of the weight by 0.94 gives an approximation of the content of anhydrous morphine.

Stage V. Titration of the morphine

27. Add 15 cc methanol to the residue, put a watch glass over the dish, and heat on the steam bath until the hot methanol has dissolved all the morphine. If necessary, break up any hard lumps with a stirring rod. (Occasionally, dispersed particles of impurities remain undissolved.) Add 4 or 5 drops methyl red indicator solution, and run in standard tenth-normal H_2SO_4 , stirring with a glass rod. Note the approximate point at which the solution becomes permanently red; add 5 to 10 cc of tenth-normal H_2SO_4 in excess. See that all the residue is dissolved as completely as possible. If necessary, heat again to be sure all the morphine is in solution. (Especially with heating, which is not used unless necessary, there may be a little brownish precipitate of some impurity.) Dilute to about 100 cc with CO_2 -free distilled water (recently boiled). Back-titrate with tenth-normal NaOH to disappearance of the red colour of the indicator. Ascertain that one drop, or if necessary two drops, of the tenth-normal H_2SO_4 will restore a slight red tint to the solution. This final acid is included in the acid reading. Subtract the amount of alkali used from the amount of acid used. Multiply the difference - the cc of tenth-normal acid used for neutralization of the morphine - by .0285 to obtain the weight of anhydrous morphine. (To calculate as crystalline morphine, multiply by .0303.) To convert to percentage, multiply by 100 and divide by the number of grammes of opium extracted for morphine (4 in this case).

IV. NOTES

Stage I. Extraction of the opium

28. In the "International Method" the opium is ground up with lime and water, rinsed into a flask and shaken for the aqueous extraction. In order to calculate an exact aliquot, determinations are made for moisture and for the extractives in the lime solution. The morphine is precipitated by adding NH_4Cl to the lime solution. The morphine titrated is equivalent to only about 2.5 grammes of the opium. A large arbitrary correction is added.

/29. In the

29. In the method of the British Pharmacopoeia, the opium is ground up with water and the lime added to the mixture. A volume allowance is made for extractives. The morphine is precipitated by adding NH_4Cl to the lime solution. The morphine titrated is equivalent to 5 grammes of the opium. A correction is added for morphine remaining in solution.

30. In the method of the United States Pharmacopoeia a total extraction is first made with water. The filtered extracts are concentrated by evaporation, lime added, and a theoretically exact aliquot obtained. The morphine is precipitated by adding NH_4Cl to the lime solution. The morphine titrated is equivalent to 4 grammes of the opium. No correction is added. It is claimed that codeins and possibly other titratable alkaloids, precipitating with the morphine, are just about equivalent to the morphine remaining in solution, in the usual opium. However, it is generally conceded that the U.S.P. results are too low on opiums that are low in morphine.

31. Stage I of the present method is on the whole most like the B.P. method. The International and U.S.P. methods both involve considerable trouble to obtain an aliquot, which does not seem justified by the over-all accuracy in either case. Total extraction is also desirable but there is no absolute proof that all the morphine of the opium is finally in solution in the lime water, in the U.S.P. method. The B.P. method formerly allowed 1 cc for extractives from 5 grammes opium, now allows 2 cc for extractives from 5 grammes. The investigations of the Secretariat have been conducted upon opiums in the air-dried condition; the conservative allowance of 1 cc for extractives from 4 grammes of opium is considered satisfactory for these tests.

32. In all lime methods it is important that the lime be very near full strength. Because of insistence of a number of chemists on this point, the United States Pharmacopoeia formerly required the use of freshly-slaked lime, but this is no longer stated. The directions for the International Method include a method for testing the lime. (Bulletin of the Health Organization of the League of Nations, Vol. VII, Extract No. 6.) The usual $\text{Ca}(\text{OH})_2$ for chemical purposes has generally seemed satisfactory in the present investigations.

Stage II. Extraction of the alkaloids from solution

33. Morphine is not highly soluble in ether-acetone. The great advantage of the new solvent combination is in extracting so little material besides the alkaloids. In spite of the fact that water is gradually taken out of the

/aqueous layer

aqueous layer into the solvent layer and the aqueous column sometimes goes far down in the extractor before the morphine is all out, very little colour and very little of other impurities are extracted, in comparison with the quantities taken out by other solvents or combinations capable of extracting morphine. Ether-acetone requires a rather close adjustment of the pH for sufficiently rapid removal of the morphine; this is accomplished in the present method. The NH_4Cl added may be varied from 1.5 to 2.0 grammes.

Stage III. Separation of the other alkaloids

34. Codeine could, of course, be separated from the morphine more easily by shaking out from an alkali solution; but then extraction from dichloroacetic acid solution would still be required to remove the "alkaloid 3". It may be mentioned that the other alkaloids are even more readily extracted by chloroform from trichloroacetic acid solution than from dichloroacetic; but too much morphine is then carried along.

Stage IV. Final extraction of the morphine

35. A number of other alcohols can be used instead of isobutyl, and some further experiments with others will still be made. Tests resembling trace reactions for morphine persist for a number of shakeouts with Marquis' and Froehde's reagents, particularly with certain kinds of opium, after the Mecke reaction has become negative (no green colour). It is not even certain that these persisting reactions are due to morphine, and collection of additional extracts has added only an insignificant titration with fiftieth-normal acid (equivalent to less than 0.1 cc of tenth-normal). Therefore the cut-off in this stage is made with Mecke's reagent.

Stage V. Titration of the morphine

36. There are some coloured substances in the final residue, with greater discoloration in some cases than others, but it is fairly easy to make the titration within one drop, or at any rate within 0.1 cc, of tenth-normal acid.

V. POSSIBILITIES OF SUBSTANTIAL ERROR

Possibilities of negative error

37. Aside from spillage or an outright mistake in reading or recording some weight or volume, there seem to be three major possibilities of negative error (too low results). These are:

(1) Incomplete

- (1) Incomplete extraction of the morphine from the opium by lime solution.
- (2) Oxidation of morphine while in the lime-water solution or during the rather long extraction with ether-acetone and the subsequent evaporation of the solvent along with extracted water.
- (3) Accumulation of small losses without any noticeable spillage.

38. The first of these possibilities is shared with any other method of lime-water extraction; indeed to some extent with any method of extraction. The new method in its subsequent stages provides for the first time a sound basis for comparing different methods of extracting the opium, but the working out of details is necessary.

39. The second possibility is believed not to occur, but this is not quite certain. There are other compounds in an opium solution even more easily oxidized than morphine, which protect the morphine from oxidation to a great degree.

40. That the third possibility may be sufficiently serious, is shown by difficulty in exactly checking a result by re-extraction of the morphine and retitration. The method requires some practice and experience; but this is probably true also of all other methods of opium assay.

Possibility of a positive error

41. Again aside from a mistake, the only major possibility of a positive error would seem to lie in the possibility of the extraction of some other titratable base. Obviously, such a base would have to be capable of being set free by ammonia and yet strong enough to be titrated, and at the same time not be capable of extraction from alkali. Although extraction from alkali is not used in the method, it can be applied after the titration, and it is found that the material finally present, extractable by chloroform from alkali solution, consists chiefly of residual traces of codeine and should not yield a titration of more than 0.5 cc of fiftieth-normal acid (0.1 cc of tenth-normal acid). In addition, such a hypothetical compound could not be very volatile nor highly soluble in water, nor could it be extracted by chloroform from dichloroacetic acid solution. There is no present evidence for the existence in opium of any basic compound meeting all these qualifications, except the morphine.

Conclusion: It is unlikely that there is any possibility of a substantial positive error in the new method.

/VI. DETERMINATION

VI. DETERMINATION OF "ALKALOID 3" AND OF MORPHINE CARRIED PAST
THE ACID SOLUTIONS

42. Shake the alkali solutions from Stage III (part III, paragraphs 18-21) with a few portions of fresh CHCl_3 , until the extractable alkaloids have been shaken out and a test on the residue from a small portion of the CHCl_3 gives no green colour, or at most a negligible one, with Mecke's reagent. Then acidify the second alkali solution with dichloroacetic acid and pour it back into the first. Substitute 5 cc water with a few drops of dichloroacetic acid in this separatory. Add more dichloroacetic acid to the first alkali solution until it is acid. In two more separatories put 5 cc dilute NH_4OH solution and 5 cc water respectively.

43. Shake out with CHCl_3 , 20 to 15 cc at a time, shaking each portion first with the main dichloroacetic acid solution, then with the acid wash water, then with the ammoniacal solution, and lastly with the last wash water. Immediately after each shaking with the ammoniacal solution, test with a piece of moistened red litmus at the mouth of the separatory, to be sure that this solution remains ammoniacal, since some dichloroacetic acid is carried along by the CHCl_3 .

Filter the extracts into a dish. Evaporate a small portion of a CHCl_3 extract for test with Froehde's reagent. With "alkaloid 3" this yields a gray-green-blue colour, which on standing becomes an intense violet. Mecke's reagent yields a bright green which on standing becomes an intense violet; this is also characteristic but the Froehde reaction is the one to watch for any indication that morphine is beginning to come out instead of the "alkaloid 3" (purple-red colour replacing or preceding the first colour due to "alkaloid 3"). If the final wash solution becomes strongly coloured during the shake-out, pour it back into the ammoniacal solution and replace with 5 cc fresh water. Continue until the test for "alkaloid 3" being extracted is negligible. About 6 extractions are required.

44. Evaporate off the CHCl_3 . Weigh the residue if desired. Dissolve it in a little methanol, add recently boiled water, and titrate with fiftieth-normal acid, using methyl-red indicator. A purer residue could be obtained as follows; Shake the CHCl_3 extracts of paragraph 43 with alkali solution, thus returning the "alkaloid 3" to alkali solution. (The CHCl_3 can be saved for redistillation.) Then wash the alkali solution with a little CCl_4 , acidify it with HCl or $(1 + 3) \text{H}_2\text{SO}_4$, make ammoniacal, and shake out the "alkaloid 3"

/with CCl_4 .

with CCl_4 . The "alkaloid 3" may possibly be codamine, but this is far from certain; it may not be among the named alkaloids of opium.

45. When the "alkaloid 3" has been shaken out of the dichloroacetic acid solutions, discard the solutions in the third and fourth separatories; pour the acid wash solution back into the main solution, replace with 3 cc water, make ammoniacal, pour this back into the main solution, and replace with 5 cc water. Make the main solution ammoniacal and shake out the morphine, if any appreciable amount is present, with portions of chloroform-ethanol solution (85 + 15). Shake each chloroformic solution with the wash water, then filter into a dish. Evaporate off the CHCl_3 , weigh the residue if desired, dissolve in a little methanol, add recently-boiled water, and titrate with fiftieth-normal acid, using methyl red indicator.

46. Thus far it seems that the percentage of "alkaloid 3" does not vary much in different types of opium. The titration is about 2.0 - 2.5 cc of fiftieth-normal acid. In methods which utilize only a shake-out from alkali solution to separate the other alkaloids from morphine, this titration is included with that of morphine and means a positive error equivalent to about 0.3% of the opium. This titration is probably not wholly due to a single base, the "alkaloid 3", but it seems that most of it is.

47. The morphine that gets as far as the alkali solution is usually negligible, and its determination is chiefly important in allowing the analyst to check his technique. Neither the morphine extracted past the acid solutions, nor the non-morphine alkaloids remaining in the acid solutions, should show as much titration as 0.5 cc of fiftieth-normal acid.

VII. DETERMINATION OF CODEINE

48. The chloroform extracts from Stage III of the Method (part III, paragraphs 15-21) are used. In each of two separatory funnels put 5 cc of dilute H_2SO_4 (2 cc concentrated H_2SO_4 per 100 cc solution). Shake the combined chloroform solutions with first one, then the other, of these two solutions. (If the volume of the chloroform is too much for a separatory funnel at one time, it can be put through in two portions.) Run the chloroform solutions back into the flask in which they were collected.

49. Pour the acid solution in the second separatory funnel into the first one, then replace with 5 cc more of dilute H_2SO_4 (2 vol.%). Add 2 or 3 drops of
/concentrated

concentrated NH_4OH to the chloroform solution and shake. Test with a piece of litmus to see that the chloroformid solution is now ammoniacal. Then shake it out with the 5 cc of 2% H_2SO_4 in the second funnel. The chloroform is now reserved for redistillation.

50. Wash the aqueous solutions with two portions of CCl_4 of about 12 cc each. Discard this CCl_4 as it is mixed with CHCl_3 . Then buffer the aqueous solutions, the first with 4 grammes and the second with 2 grammes of sodium acetate.

51. Shake out the thebaine with CCl_4 . Continue as in the usual separation of thebaine and cryptopine from codeine (E/CN.7/202, paragraphs 10 and 11): Evaporate portions of the CCl_4 and test for thebaine with (2 + 1) H_2SO_4 . When the thebaine is nearly out, check on extraction of cryptopine with fuming H_2SO_4 (15% free SO_3), and on whether any codeine is coming out, with "(3 + 1)" Marquis' reagent (4 drops HCHO , 3 cc H_2SO_4 , 1 cc H_2O). Continue the extraction to a negligible thebaine reaction, but do not try to get the rest of the cryptopine out if it involves loss of codeine. If the CCl_4 test portions are evaporated on the same steam bath where the fumes may affect alkaloidal residues for titration, add a drop of ammonia to each before evaporation to neutralize any acetic acid. The CCl_4 (aside from the test portions) can be saved for redistillation.

52. Continue as in the usual shake out of codeine (E/CN.7/202, paragraph 12). Make the second solution alkaline and pour it back into the first; make the first solution alkaline with an excess of NaOH . Shake out with CCl_4 past three washes: 5 cc alkali solution (second separatory), 5 cc water containing a little ammonium salt and ammonia (third separatory), and a 10 cc wash of water (fourth separatory). Test for completion of the extraction of codeine from each solution in turn by evaporating a small portion of the CCl_4 and testing the residue with Marquis' reagent (2 drops HCHO in 3 cc H_2SO_4).

53. Filter the extracts through a little cotton into a glass dish and evaporate off the solvent. Dry the residue on the steam bath to complete crystallization; cool and weigh. Multiplication by 0.94 gives an approximation of the anhydrous codeine. Dissolve the residue in a little methanol, add 2 or 3 drops methyl red indicator solution, and titrate with fiftieth-normal H_2SO_4 , adding CO_2 free (recently boiled) water to obtain the end-point. One cc $n/50$ acid = .00599 grammes codeine (anhydrous). Since the extraction represents

/4 grammes

4 grammes opium (instead of 2 grammes; as in E/CN.7/202), multiply the result by 100 and divide by 4 for the codeine percentage of the opium as determined on the lime-water extract.

VIII. COLOUR DETERMINATION OF "PORPHYROXINE-MECONIDINE"

54. The chloroform extracts from Stage III of the Method (paragraphs 15-21) may be used for the determination of "porphyroxine-meconidine" instead of codeine. In three separatory funnels put 20, 10 and 10 cc of dilute H_2SO_4 (2 volumes of concentrated H_2SO_4 to 100 of solution). Shake the chloroform extracts with the H_2SO_4 solutions in turn, then combine the H_2SO_4 solutions in the first separatory, pour out a portion of the solution in a test tube and heat in the steam bath to full development of the red colour. Since the "porphyroxine-meconidine" from 4 grammes of opium is in 40 cc aqueous solution, the red colour corresponding to the values previously given may be read on the Lovibond slides with the solution in the 1/8 inch cell, or calculated to correspond to this reading. (See E/CN.7/195; and E/CN.7/202, part VI.)

55. In case it is desired to determine both codeine and "porphyroxine-meconidine", the best way, probably, is to wash the $CHCl_3$ extracts as they are collected, first with water containing a little ammonium salt and a drop of ammonia, and then with plain water; and then evaporate off the $CHCl_3$ and proceed with the residue as in E/CN.7/202, parts IV and VI.

56. It may be mentioned that the chloroform extracts also contain the other phenolic alkaloids (in addition to the "porphyroxine-meconidine") that are extractable by chloroform from alkali. However, a suitable determination has not yet been worked out.

IX. CONCLUSIONS

57. The new features of the new morphine method are the continuous extraction of the alkaloids by ether-acetone and the separation of the other alkaloids from morphine by shaking them out from dichloroacetic acid solution with chloroform.

58. In Stage I, (paragraph 11) a lime-water solution of the opium is obtained, as in the present precipitation methods. However, the solvent-extraction method has the advantage that the volume of opium solution need not be so limited, and any of the various methods of obtaining the lime-water solution (paragraphs 28-30) could be used. Furthermore, subsequent stages of the new method are not, in principle, dependent on the use of a lime-water extract, and eventually other methods of extracting the opium can be explored (paragraph 9).

59. In Stage II (paragraphs 12-14) continuous extraction with ether-acetone meets a number of objections that have been raised against other extractive methods (paragraphs 5, 6). The great advantage of the new solvent-combination is in extracting so little colour and other extraneous material besides the alkaloids (paragraph 33).
60. In Stage III (paragraphs 15-20), an entirely new method, more successful than any used hitherto (paragraphs 7, 8), is applied to separating the other alkaloids from morphine. This is extraction with chloroform from dichloroacetic acid solution. This separates even the minor phenolic alkaloid which presumably stays with morphine in previous extractive methods (paragraphs 7, 46), carrying along only a negligible amount of morphine (paragraph 47), and leaving behind only a negligible amount of titratable alkaloids extractable by chloroform from alkali solution (paragraph 41).
61. In Stage IV (paragraphs 21-26), as in Stage II, the extraction of morphine is made complete by test. Although the morphine recovered is somewhat brown, it crystallizes completely and the weight, as crystalline morphine, usually corresponds very closely to the titration.
62. In Stage V (paragraph 27) the extracted morphine is titrated with standard acid. For such titrations, an uncertainty as to the exact reading of about 0.1 cc is normal. Every effort has been made to keep any possible error, either positive or negative, within this same limit, and so far as can be ascertained, this has been done (paragraph 47), at least from Stage II onward (paragraph 1).
63. The equivalent of 0.1 cc of tenth-normal acid is 2.85 milligrammes of morphine, or 0.07% of the opium. It is hoped, therefore, that any error from Stage II onward can be kept within about 0.1% of the opium. However, this requires practice and experience (paragraph 40), and the possibilities of greater error, particularly any arising from Stage I (paragraph 38), should not be forgotten (part V, paragraphs 37-41).
64. The method as a whole utilizes the advantages of extractive methods over precipitation methods (paragraph 4), while meeting the principal objections that have been brought against previous extractive methods (paragraphs 5-8). The method is highly flexible and adaptable; no minimum quantity of morphine is necessary, and it can be applied, with no more than obvious modifications, to most kinds of preparations or mixtures of morphine or opium.

/65. The method

65. The method sets a new standard for the assay of opium. It can be used to determine the accuracy of the pharmacopoeial methods (which may seem to have some advantage in simplicity) in the following ways:

- (a) The final results can be compared.
- (b) The other alkaloids precipitated and titrated with the morphine in a pharmacopoeial method, can be separated as in Stage III of the new method, and determined.
- (c) The morphine remaining in the lime-water filtrate in a pharmacopoeial method, can be determined by the new method, starting with Stage II.
- (d) The lime-water extracts prepared by different methods can be accurately compared for content of morphine, by means of the new method.

The new separation of the other alkaloids from morphine can also be used to determine whether there is any important error in previous extractive methods, and to modify them so as to avoid a positive error. It is hoped that all these comparative studies will soon be made by international collaboration.